Anal. Chem. **1997,** *69,* 364−372

Calibration of a Commercial Solid-Phase Microextraction Device for Measuring Headspace Concentrations of Organic Volatiles

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Solid-phase microextraction (SPME) is a versatile new technique for collecting headspace volatiles prior to GC analysis. The commercial availability of uniform SPME fibers makes routine, practical quantitation of headspace concentrations possible, but straightforward information for relating GC peak areas from SPME analyses to headspace concentrations has not been available. The calibration factors (amount absorbed by the fiber divided by headspace concentration) were determined for 71 compounds using SPME fibers with a 100 μm poly(dimethylsiloxane) coating. The compounds ranged from 1 to 16 carbons in size and included a variety of functional groups. Calibration factors varied widely, being 7000 times higher for tetradecane than for acetaldehyde. Most compounds with a Kovats retention index of <1300 on a nonpolar GC column (DB-1) equilibrated with the fiber in 30 min or less. A regression model is presented for predicting the calibration factor from GC retention index, temperature, and analyte functional class. The calibration factor increased with retention index but decreased with increasing sampling temperature. For a given retention index, polar compounds such as amines and alcohols were absorbed by the fibers in greater amounts than were hydrocarbons. Henry's law constants determined using SPME were in general agreement with literature values, which supported the accuracy of the measured calibration factors. An unexpected concentration dependence of calibration factors was noted, especially for nitrogen-containing and hydroxy compounds; calibration factors were relatively higher (the SPME fiber was more sensitive) at the lower analyte concentrations.

Solid-phase microextraction (SPME) is a simple and versatile technique for sampling of volatile organic compounds. It was originally developed for sampling from aqueous solutions,[1] but it has also been used for analyzing headspace volatiles,[2,3] an application of particular interest to biologists and chemical ecologists. The compounds are absorbed by the polymeric coating of a slender fused silica fiber. The fiber is then withdrawn into the needle of the syringe-like SPME device, and the needle is inserted through the inlet septum of a gas chromatograph. Finally, the fiber is extended again in the hot injector to desorb the sample. The sample is collected and analyzed (by GC or GC/MS) without using solvents and without sample preparation or handling.

SPME has obvious utility for qualitative analysis of natural volatiles from plants, fungal cultures, insects, and other sources, but quantitation of these volatiles is not trivial. The SPME fibers are not uniformly sensitive to all compounds, and therefore, relative GC peak areas for an SPME sample do not properly reflect the true proportions of the components in the headspace. Furthermore, other factors such as sampling time and temperature can affect quantitation.[2,3]

Uniform SPME fibers are now commercially available, and there is a practical need for calibration information so that headspace concentrations can be calculated directly from GC detector responses. By theory, SPME is an equilibrium process, and at equilibrium the concentration of an analyte in the fiber coating is directly proportional to its concentration in the headspace.[2] Because the volume of the fiber coating is constant in the commercial fibers, the coating volume (which might be impractical to measure) no longer has to be included in calibration calculations; the theory simplifies to the absolute *amount* of analyte absorbed by the fiber being directly proportional to headspace concentration. In this report, the proportionality constant will be called *K*, the "calibration factor".

In this study, a simple system involving only gas-phase analytes and the SPME fiber was used to investigate quantitative properties of the fiber. Following an initial evaluation of sampling and GC injection procedures, calibration factors were measured for 71 compounds of diverse functionality and ranging in size from 1 to 16 carbon atoms. Then a regression model was developed for predicting calibration factors from readily available chemical, physical, and chromatographic information; the model allows quantitation by SPME even when calibration factors have not been experimentally determined.

EXPERIMENTAL SECTION

SPME Device. The SPME fiber coating was 100 μm of poly(dimethylsiloxane), obtained from Supelco (Bellefonte, PA). The fibers were conditioned for at least 5 h at 200 °C before first experimental use; fibers were reused until accidental breakage occurred. Usually, >100 injections were made with each fiber.

Standard Solutions. Eleven analysis mixtures were prepared as listed in Table 1. Components of a mixture were chemically compatible and separable by GC. Aldehydes were freshly distilled to remove traces of trimer. Components were measured gravimetrically; initial solutions were nominally 1 mg/mL (1 μg/μL) in 25-mL volumetric flasks. The solvent was always methylene

(1) Arthur, C. L.; Pawliszyn, J. *Anal. Chem.* **1990**, *62*, 2145−2148.
(2) Zhang, Z.; Pawliszyn, J. *Anal. Chem.* **1993**, *65*, 1843−1852.
(3) Chai, M.; Pawliszyn, J. *Environ. Sci. Technol.* **1995**, *29*, 693−701.

chloride except for mixture 6, for which the solvent was methanol. Then two consecutive 1:10 dilutions were made (nominally 100 and 10 ng/μL). Aliquots of these two dilutions were transferred to GC autosampler vials for subsequent GC analysis. The remainders of all three solutions were transferred to vials with Teflon caps and stored in a freezer. Solutions were prepared just before they were needed.

Gas Chromatography. A Hewlett-Packard 5890 Series II GC was used for all analyses. The instrument was equipped with on-column and split/splitless injectors, flame ionization detector (FID), autoinjector, and Hewlett-Packard 3396A integrator. The column was a 30-m DB-1 capillary (J&W Scientific, Folsom, CA) having an inside diameter of 0.32 mm and a film thickness of 5.0 μm. A 10-cm piece of deactivated fused silica tubing (0.5 mm i.d.) was attached to the inlet end of the column with a glass press-fit connector so that the SPME device could be used with the on-column injector. The carrier gas was helium at a head pressure of 16.5 psig. The detector temperature was 250 °C.

SPME injections were done manually through the on-column injector. The injector temperature was 200 °C. The oven temperature program was 50 °C for 1 min, followed by an increase to 250 °C at 10 °C/min and a final 7-min hold at 250 °C. The split/splitless injector (at 200 °C) was used for conditioning the fiber between analyses; the injector outlet was capped, but the split valve was always open and helium was passed through (5 psig) so that the injector was constantly purged.

FID response factors were determined for the analytes by using the autosampler and on-column injector within 1 day of analyzing the corresponding gas sample by SPME. The diluted solutions in autosampler vials described above (nominally 100 and 10 ng/μL) were each analyzed five times. The autoinjection volume was 0.50 μL. The oven temperature program began at 35 °C for samples in methylene chloride and 60 °C for methanol; these starting temperatures were chosen to optimize peak form. The temperature was held at the initial value for 1 min and then increased to 250 °C at 10 °C/min. For these analyses, the injector was programmed to be 3 °C warmer than the oven temperature. The amount of each compound injected was calculated from the weight in the original solution, the dilution factors, and the 0.50 μL injection volume. Response factors were calculated as nanograms per integrator area unit. Generally, the response factors for the two dilutions were very similar, and the overall mean was used. With some compounds such as amines, the lower concentration gave systematically higher response factors; this was usually due to the difficulty of integrating tailing peaks. In such cases, only the values for the higher concentration were used because these standards matched the SPME injections in GC peak shape more closely than the less concentrated standards did. Relative response factors were calculated using pentadecane as the comparison standard (Table 1). The absolute response factor for pentadecane was 3.20×10^{-4} ng/area unit.

Kovats retention indexes were calculated for the compounds using a program of 35 °C for 1 min, then 10 °C/min to 270 °C. Standards were *n*-alkanes of 3−16 carbons, with propane and butane being injected by SPME. Retention indexes were calculated by linear interpolation[4] and are listed in Table 1.

Gas Samples and Experiments. Experimental methods were patterned after those of Chai and Pawliszyn.[3] Gas samples were prepared in bottles of known volume, approximately 1 L. Each bottle was silanized with dichlorodimethylsilane, had a screw-top lid with a Teflon-lined septum, and contained a Teflon-coated magnetic stirring bar. These precautions were to reduce adsorption of analytes on bottle walls and to prevent settling of dense organic vapors. In preliminary work, bottles without silanized surfaces or magnetic stirrers gave low and erratic results, especially for compounds of higher molecular weight or polarity.

In general, analyte samples in a small amount of solvent (10 or 25 μL) were introduced into the sealed sample bottles (which contained ambient laboratory air); all liquid evaporated. The bottles were then placed in an incubator at the experimental temperature (±0.5 °C) for at least 1 h before SPME sampling began. For sampling, a freshly conditioned SPME fiber was passed through the septum into the bottle (set upright) and kept there for the appropriate sampling period. The magnetic stirrer was operated throughout sampling. The SPME injection was then made immediately into the GC, after which the SPME device was moved to the conditioning injector for 2−5 min prior to acquisition of the next sample. Experimental variables were sampling time, injection time, analyte concentration, and sampling temperature.

The first experiment was a preliminary study that explored the effect of sampling time on GC peak area. Initially, only mixture 4 was used (Table 1). A 10-μL aliquot of the undiluted mixture was added to each bottle. The bottles were sampled at 25 °C for 1, 3, 10, 30, or 100 min (two replications for 3 and 10 min, one for the other times). The injection time was 0.5 min. Subsequently, 30- and 100-min sampling times were compared for the analytes in Table 1 not contained in mixture 4.

The second experiment was another preliminary study that examined the effect of injection time on GC peak area; only mixture 4 was used. To each bottle was added 10 μL of the undiluted mixture. All bottles were sampled for 30 min at 25 °C, but the injection times were 0.05, 0.15, 0.5, 1.5, or 5 min. There were two replications for each injection time.

The third experiment was the major part of the research in which calibration factors were determined for 71 analytes. Sample bottles were prepared for each mixture (Table 1) at four gas-phase concentrations. Aliquots of 10 or 25 μL of the undiluted mixture (nominally 1 μg/μL) or 10 or 25 μL of the first dilution (nominally 100 ng/μL) were added to bottles. Thus, the four gas-phase concentrations were nominally 1, 2.5, 10, and 25 μg/L for each component. There were at least two replications for each concentration. Sampling was at 25 °C for 30 min; the injection time was 0.5 min. For each mixture, the concentrations were analyzed in a random order. It was expected that all headspace concentrations would provide the same value for K for an analyte unless problems such as adsorption by bottle walls occurred.

The fourth experiment determined the effect of sampling temperature on calibration factor. Five gas samples were prepared for each mixture (Table 1), using 10 μL of the undiluted mixture (about 10 μg per component) in all cases. These were sampled in incubators, two at 15 °C, two at 35 °C, and one at 25 °C. The 25 °C sample was to verify consistency with the third experiment. The sampling time was 30 min, and the injection time was 0.5 min.

In the final experiment, Henry's law constants were determined using SPME for measurement of headspace concentrations. Aqueous solutions (Table 3) were prepared gravimetrically, and 10-mL aliquots were introduced into 27-mL crimp-top autosampler

(4) Poole, C. F.; Schuette, S. A. *Contemporary Practice of Chromatography*; Elsevier: Amsterdam, The Netherlands, 1984; pp 23−25.

Table 1. Summary of Measured Calibration Information, Fitted Values from Regression Model, GC Detection and Retention Parameters, and Composition of Analysis Mixtures Involving 71 Standard Compounds

compound	measd K (mL)	measd log(K)	SE (N)	fitted log(K)	rel resp factor[a]	Kovats retention index	analysis mixture
			Hydrocarbons				
pentane	0.042	−1.38	0.019 (14)	−1.36	1.00	500	6
hexane	0.105	−0.98	0.017 (13)	−0.94	1.03	600	3
nonane	2.24	0.35	0.008 (13)	0.31	1.06	900	7
decane	5.89	0.77	0.008 (13)	0.73	1.04	1000	7
undecane	15.5	1.19	0.012 (13)	1.15	1.02	1100	7
dodecane	38.9	1.59	0.014 (13)	1.57	1.00	1200	7
tridecane	83.2	1.92	0.017 (13)	1.99	1.03	1300	7
tetradecane[b]	132.	2.12	0.035 (9)	2.41	1.01	1400	7
pentadecane[b]	110.	2.04	0.026 (24)	2.83	1.00	1500	5, 6, 7
hexadecane[b]	57.5	1.76	0.064 (9)	3.25	0.98	1600	7
			Esters				
ethyl acetate	0.186	−0.73	0.016 (11)	−0.74	2.27	597	1
propyl acetate	0.437	−0.36	0.016 (11)	−0.33	1.92	695	4
ethyl isobutyrate	0.692	−0.16	0.016 (13)	−0.13	1.64	743	4
isobutyl acetate	0.891	−0.05	0.013 (13)	−0.07	1.64	756	4
butyl acetate	1.29	0.11	0.013 (13)	0.09	1.59	794	4
isopentyl acetate	2.34	0.37	0.014 (13)	0.36	1.59	858	4
pentyl acetate	3.55	0.55	0.008 (13)	0.50	1.54	893	1
ethyl hexanoate	7.08	0.85	0.014 (13)	0.86	1.47	979	4
benzyl acetate	26.9	1.43	0.015 (13)	1.37	1.27	1147	4
ethyl octanoate	42.7	1.63	0.015 (13)	1.69	1.37	1178	1
2-phenylethyl acetate	55.0	1.74	0.013 (17)	1.77	1.33	1244	2
ethyl decanoate[b]	126.	2.10	0.024 (9)	2.32	1.41	1377	4
			Ketones				
acetone	0.074	−1.13	0.023 (21)	−1.13	2.00	471	3, 6
2-butanone	0.148	−0.83	0.025 (12)	−0.83	1.45	575	4
2-pentanone	0.372	−0.43	0.018 (13)	−0.44	1.43	666	4
2-hexanone	1.00	0.00	0.017 (13)	0.01	1.25	770	4
2-heptanone	2.51	0.40	0.014 (22)	0.42	1.28	872	2, 10
			Aldehydes				
acetaldehyde	0.015	−1.82	0.034 (19)	−1.72	2.63	359	9
propanal	0.069	−1.16	0.027 (19)	−1.25	2.27	472	9
butanal	0.132	−0.88	0.025 (19)	−0.81	1.89	575	9
isopentanal	0.398	−0.40	0.020 (19)	−0.55	2.04	639	9
(E)-2-methyl-2-butenal	0.562	−0.25	0.010 (15)	−0.19	1.47	724	6
hexanal	1.35	0.13	0.011 (19)	0.06	2.38	783	9
benzaldehyde	3.39	0.53	0.008 (17)	0.56	1.18	950	2
octanal	6.46	0.81	0.006 (15)	0.91	1.72	987	6
			Alcohols				
methanol	0.059	−1.23	0.033 (25)	−1.23	2.86	361	2, 3, 10
ethanol	0.087	−1.06	0.018 (26)	−1.06	2.08	436	1, 3
1-propanol	0.141	−0.85	0.018 (26)	−0.78	1.61	546	2, 6
2-butanol	0.224	−0.65	0.021 (23)	−0.62	1.54	585	2, 10
2-methyl-1-propanol	0.282	−0.55	0.017 (23)	−0.51	1.33	611	2, 10
1-butanol	0.468	−0.33	0.018 (23)	−0.36	1.43	645	2, 10
3-methyl-1-butanol	0.891	−0.05	0.018 (23)	−0.06	1.35	717	2, 10
2-methyl-1-butanol	0.955	−0.02	0.023 (13)	−0.04	1.23	722	4
1-hexanol	4.17	0.62	0.016 (13)	0.50	1.41	851	1
2-heptanol	4.27	0.63	0.019 (22)	0.64	1.32	884	2, 10
1-octanol	23.4	1.37	0.012 (28)	1.35	1.30	1054	6, 7
2-phenylethanol	23.4	1.37	0.017 (17)	1.36	1.16	1105	2
1-decanol[b]	72.4	1.86	0.025 (11)	2.21	1.27	1259	6, 7
			Carboxylic Acids				
acetic acid	0.389	−0.41	0.017 (12)	−0.40	3.85	562	5
propanoic acid	1.05	0.02	0.012 (17)	0.00	2.56	659	5
2-methylpropanoic acid	1.91	0.28	0.013 (17)	0.27	2.00	723	5
3-methylbutanoic acid	3.80	0.58	0.014 (15)	0.61	1.82	804	5
hexanoic acid[b]	7.41	0.87	0.047 (6)	1.18	1.56	939	5
octanoic acid[b]	4.36	0.64	0.074 (10)	1.99	1.79	1134	5
			Amines				
diethylamine	2.69	0.43	0.059 (13)	0.22	1.45	564	8
2-methylpropylamine	2.04	0.31	0.053 (13)	0.33	1.37	591	
diisopropylamine	2.46	0.39	0.069 (13)	0.60	1.16	655	8
triethylamine	4.47	0.65	0.064 (13)	0.72	1.16	682	8
3-methylbutylamine	8.13	0.91	0.015 (10)	0.77	1.82	696	7, 11
hexylamine	22.4	1.35	0.024 (11)	1.34	1.54	830	8
cyclohexylamine	26.9	1.43	0.028 (12)	1.45	1.56	857	8
			Pyrazines and Pyridines				
pyrazine	2.00	0.30	0.038 (13)	0.31	1.56	711	4
pyridine	2.69	0.43	0.042 (13)	0.37	1.30	726	8
2,5-dimethylpyrazine	11.2	1.05	0.030 (17)	1.08	1.32	895	2

Table 1 (Continued)

compound	measd K (mL)	measd log(K)	SE (N)	fitted log(K)	rel resp factor[a]	Kovats retention index	analysis mixture
			Diols				
2,3-butanediol (chiral)	3.31	0.52	0.028 (22)	0.53	2.13	750	2, 10
2,3-butanediol (meso)	3.72	0.57	0.030 (22)	0.57	2.17	759	2, 10
			Disulfides				
dimethyl disulfide	0.40	−0.40	0.014 (12)	−0.40	3.03	736	7
			Hydroxyketones				
3-hydroxy-2-butanone	0.66	−0.18	0.010 (30)	−0.18	2.94	681	2, 6, 10
			Alkylphenols				
4-ethylphenol	30.9	1.49	0.016 (17)	1.49	1.18	1145	2
			o-Methoxyphenols				
2-methoxyphenol	11.0	1.04	0.010 (17)	0.99	1.47	1080	2
4-ethyl-2-methoxyphenol	53.7	1.73	0.015 (9)	1.82	1.37	1278	1

[a] Relative response factor equals absolute response factor for subject compound divided by absolute response factor for pentadecane. Units for absolute response factor: ng/peak area. [b] Analyte not in equilibrium with fiber.

vials (Alltech, Deerfield, IL) containing magnetic stirring bars, and the vials were sealed with Teflon-lined septa. SPME samples were for 30 min at 25 °C, and injections were for 0.5 min.

Calculations. The absolute amount of analyte absorbed by the fiber (in nanograms) was calculated by multiplying the GC peak area by the measured FID response factor. Headspace concentration at the end of the sampling time (in nanograms per milliliter) was calculated as the absolute amount of analyte introduced into the sampling bottle (minus the amount in the fiber), divided by the bottle volume. The amount of analyte absorbed by the fiber was not neglected in calculations because it ranged as high as 14% of the amount added to the bottle (with tetradecane). The calibration factor K (in milliliters) was the amount of analyte in the fiber divided by the headspace concentration in the sampling bottle at the end of the sampling period (when, for most analytes, the system was in equilibrium).

Chromatograms were inspected prior to using data for statistical calculations; 44 of the 1306 data points were rejected because electronic noise, chromatographic artifacts, or very small peaks led to dubious integrations.

Multiple regression and analysis of variance calculations were conducted with the Statistix software package.[5] All statistical calculations involving K were done after transformation to the logarithmic scale; the transformation stabilized variance and made relationships with other variables more linear.

RESULTS

Sampling and Injection. Thirty-minute SPME collections from the 1-L gas samples were sufficiently long for the fiber to equilibrate with 64 of the 71 compounds at 25 °C (Figure 1). For these there was no detectable difference in the amounts absorbed by the fiber between sampling times of 30 and 100 min. Only the highest molecular weight alkanes (tetradecane, pentadecane, and hexadecane), ester (ethyl decanoate), alcohol (decanol), and acids (hexanoic and octanoic) did not equilibrate within 30 min. For lower molecular weight compounds (e.g., propyl acetate in Figure 1), equilibrium was achieved as rapidly as within 1 min. Thus, a 30-min sampling time (chosen as a convenient complement to GC runs of about 30 min) would provide calibration factors independent of sampling time for all but seven of the test analytes.

(5) Analytical Software. *Statistix Version 4.1 User's Manual*; Analytical Software: Tallahassee, FL, 1994.

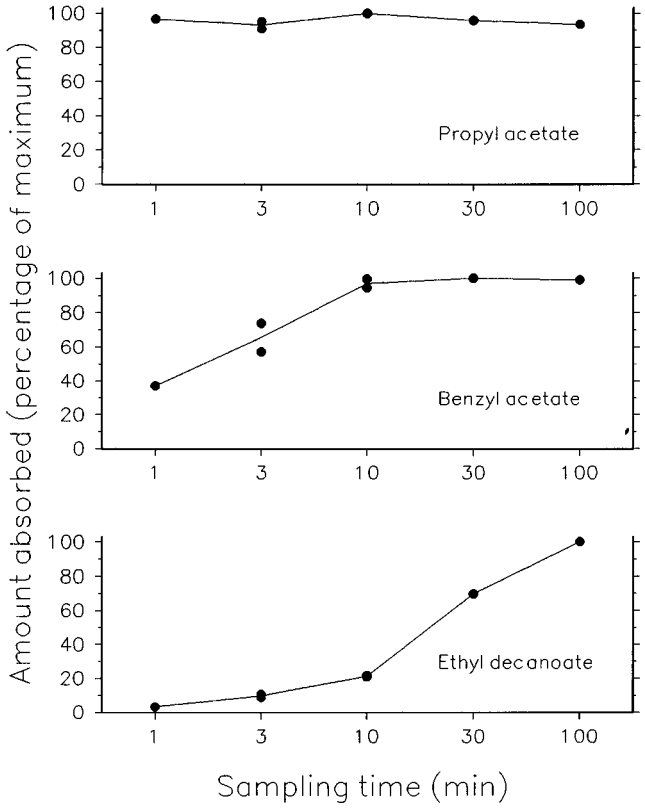

Figure 1. Absorption as a function of sampling time at 25 °C for example analytes.

The routinely used injection time of 0.5 min at 200 °C was sufficient for essentially complete desorption of the analytes tested (analysis mixture 4, Table 1). Over all compounds, desorption was 96% complete after 0.05 min, and there was no significant difference among any injection times from 0.15 to 5.0 min ($P > 0.25$, Figure 2). Thus, incomplete injection would not be a problem in determining the calibration factors.

Calibration Factors. Calibration factors (K) for the 71 analytes at 25 °C are summarized in Table 1, along with log(K) and its standard error. Data from experiments 3 and 4 were used, adjusting the 15 and 35 °C data by regression (see below). K is the amount of analyte absorbed by the fiber at equilibrium (or within the defined sampling period if equilibrium is not estab-

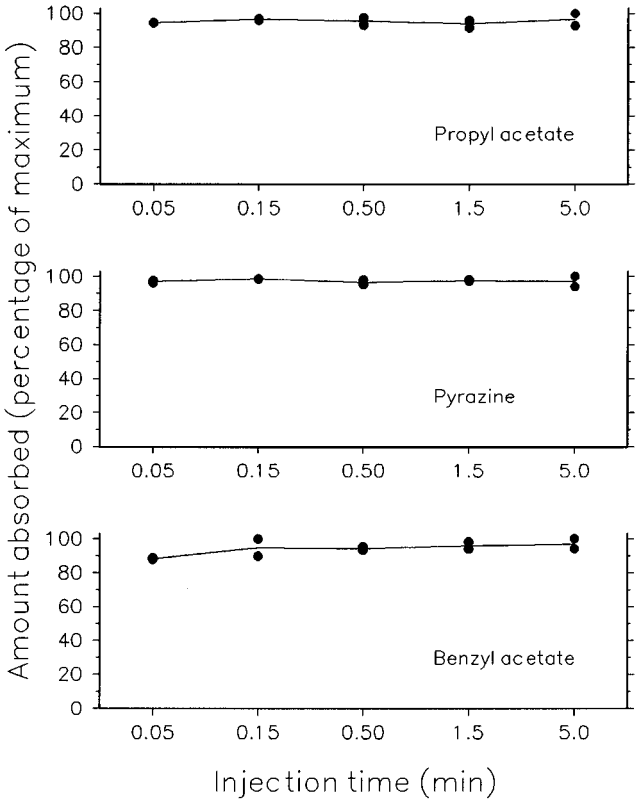

Figure 2. GC detector response as a function of injection time at 200 °C.

lished) divided by the amount per milliliter in the headspace. *K* is independent of units for amount as long as the same units are used in the numerator and denominator. A large value indicates a high affinity of the fiber for the compound. A physical meaning of an equilibrium *K* value is that if the volume of an analyte gas sample (in milliliters) is equal to *K*, exactly one-half of the analyte will be absorbed by the SPME fiber at equilibrium.

A tremendous range of values was observed; the highest (132 for tetradecane) was 7000 times greater than the lowest (0.015 for acetaldehyde). For the seven compounds not reaching equilibrium within 30 min, the observed *K* is less than the maximum possible (equilibrium) value.

Regression Analysis. From graphs, log(*K*) increased with increasing GC retention index within the alkane, ester, and alcohol series (Figure 3), but it decreased with increasing sampling temperature (Figure 4). These relationships, along with information about analyte functionality, were combined in a multiple linear regression model (Table 2). Log(*K*) can be described rather successfully if the Kovats GC retention index, sampling temperature, and functional group of the analyte are known. The model was based on 1038 determinations of *K* for the 64 analytes that equilibrate with the fiber within 30 min, and it accounted for 98.5% of the variance in the data set. Fitted values for log(*K*) are given in Table 1. A graph of observed versus fitted values appears in Figure 5.

The Kovats index was the best single predictor variable, accounting for 82% of the variance in the raw data. Overall, log(*K*) increased by 0.419 (i.e, *K* increased by a factor of 2.62) for each additional carbon unit (for each increase of 100 in Kovats index). However, *K* decreased by a factor of 0.55 for each 10 °C increase in sampling temperature. The temperature coefficients (represented by slopes in Figure 4) did vary slightly among

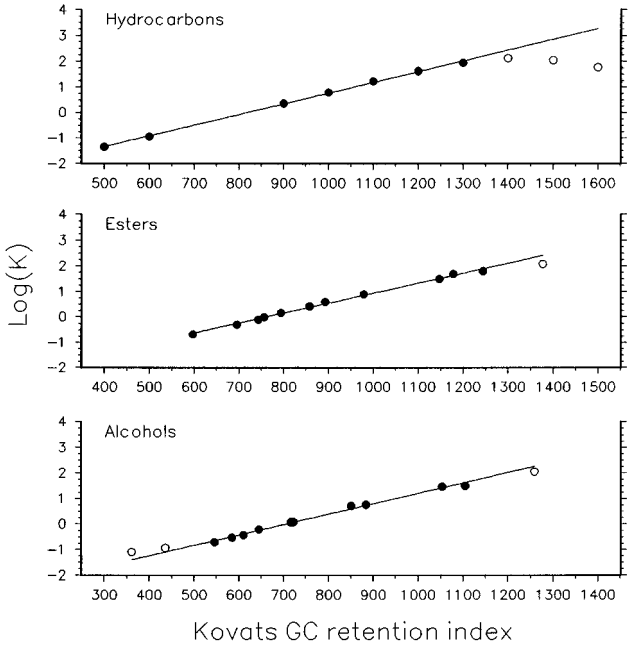

Figure 3. Relationship between log(*K*) and Kovats retention index on DB-1 phase for three functional classes. Open circles at high Kovats index represent analytes not in equilibrium with the fiber; open circles at low Kovats index are for methanol and ethanol, which deviated from the trend for other alcohols.

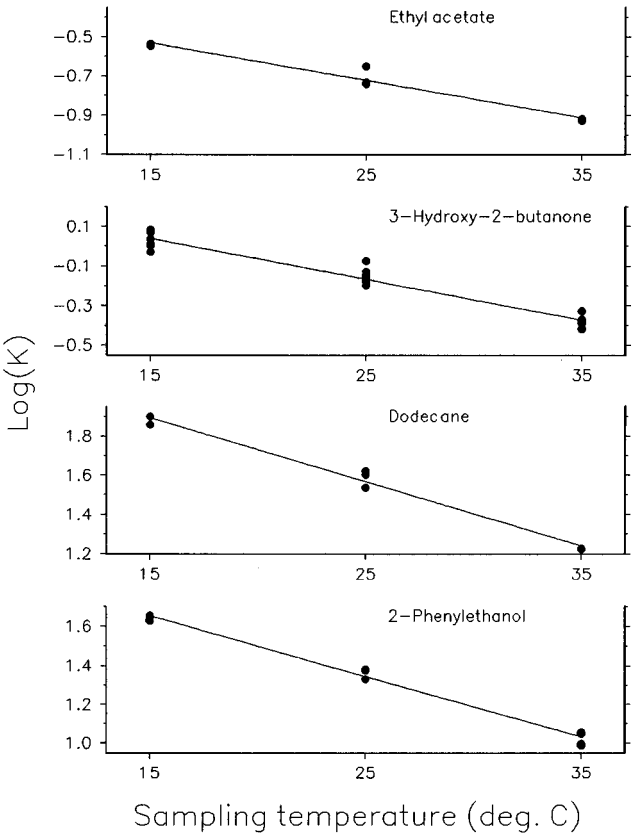

Figure 4. Relationship between log(*K*) and sampling temperature for four analytes. Headspace concentrations were always about 10 ng/mL.

compounds, which would be expected on thermodynamic grounds. However, the variability was slight, and in the interest of simplicity, approximation with just one value is believed reasonable within the range of 15−35 °C.

Table 2. Multiple Regression Model for log(*K*)

Regression Model:
$\log(K) = B_0 + B_1(R) + B_2(T) + B_3 + B_4$

R^2	0.985
residual SD	0.110
19 fitted parameters,	1019*df* for residual

Variables and Units

K	calibration factor (mL)
R	Kovats GC retention index
T	temperature (°C)

Regression Coefficients

coeff	description	value	standard error
B_0	model constant term	−2.82	0.028
B_1	factor for GC retention index	0.00419	0.000023
B_2	factor for temperature	−0.0257	0.00059
B_3	functional group correction terms		
	hydrocarbons[a]	0.00	na[b]
	o-methoxyphenols	0.12	0.029
	esters	0.22	0.015
	ketones (except acetone)	0.22	0.019
	aldehydes	0.23	0.016
	alkylphenols	0.35	0.033
	alcohols (except methanol and ethanol)	0.39	0.015
	carboxylic acids	0.70	0.019
	pyrazines and pyridine	0.79	0.021
	diols	0.84	0.021
	amines	1.32	0.017
	individual compounds		
	methanol	0.72	0.028
	ethanol	0.57	0.027
	acetone	0.36	0.029
	3-hydroxy-2-butanone	0.43	0.024
	dimethyl disulfide	−0.03	0.034
B_4	correction for benzene ring		
	ring present	−0.20	0.017
	ring absent[a]	0.00	na[b]

[a] Coefficient included in the constant term of the model. [b] Not applicable.

The value of *K* depended strongly on the functional class of the compound. For a given GC retention index, almost all functional classes were absorbed more readily than hydrocarbons. For esters and ketones, log(*K*) was 0.22 higher (*K* was 1.65× higher) than for hydrocarbons. For aldehydes, most alcohols, and acids, the factors for *K* were 1.70, 2.5, and 5.0, respectively. The 2,3-butanediol isomers had even higher affinity for the fiber, with a factor of 7.0 relative to hydrocarbons. Methanol and ethanol deviated somewhat from the higher alcohols, being 5.3 and 3.7 times more readily absorbed than hydrocarbons. Most striking was the high affinity of the fibers for nitrogen-containing compounds. As a group, amines were 21× more readily absorbed than hydrocarbons, and for heterocyclic aromatic compounds such as pyrazines and pyridines, the factor was 6.1×. In general, compounds containing a benzene ring were absorbed only 0.63× as readily as other members of the functional class without the ring. After allowing for the benzene ring effect, alkylphenols were absorbed similarly to aliphatic alcohols (factor was 2.3× instead of 2.5×). *o*-Methoxyphenols were less well absorbed (factor was 1.3×), perhaps because intramolecular hydrogen bonding would change the character of the OH group. With 3-hydroxy-2-butanone (for which the factor was 2.7×), the net tendency to absorb probably results from the presence and interaction of hydroxyl and carbonyl groups. Dimethyl disulfide behaved essentially as a hydrocarbon.

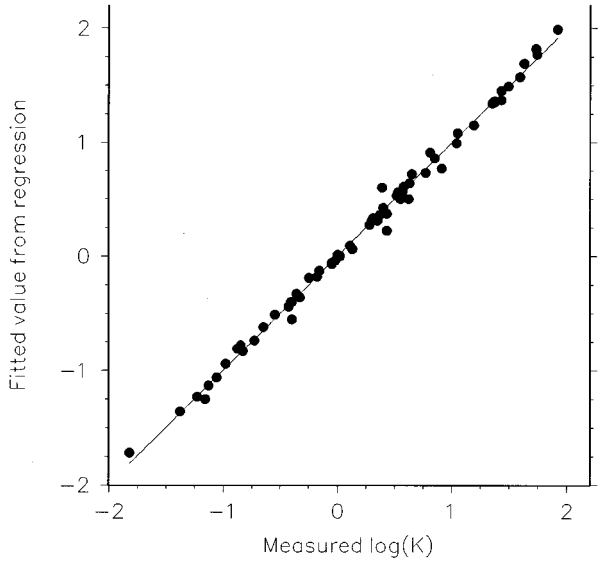

Figure 5. Performance of regression model: plot of fitted versus measured values of log(*K*). Diagonal line represents equivalence of these values.

Variability in *K*. The overall standard deviation of log(*K*) for repeated samples under identical conditions was 0.073 (592*df*), which corresponded to ±18% for *K*. This value includes variability due to the preparation of the gas samples, GC peak integration, differences among individual fibers, and differences between analysts as well as variability intrinsic to the SPME technique. Standard deviations were relatively higher for the most volatile and the most polar of the compounds because of difficulty in integrating their GC peaks (relatively small size and broad peak shape, respectively). No statistical models for log(*K*) could be expected to have a smaller residual standard deviation than 0.073.

The values for log(*K*) in Table 1 had an overall standard deviation of 0.0954 (983*df*), which corresponds to ±25% for a single measurement of *K*. The regression model had a residual standard deviation of 0.110 (1019*df*), corresponding to ±29% variation for a single measurement of *K*. The values for log(*K*) had a higher standard deviation than 0.073, primarily because log(*K*) was dependent on concentration (as described below). The residual standard deviation from the regression model reflected this concentration dependence as well as some lack of fit (difference between observed means and fitted values, Figure 5 and Table 1).

Concentration Dependence. Although no dependence of log(*K*) on headspace concentration was expected from theory, concentration dependence was nevertheless evident in many cases (Figure 6). When allowance for concentration dependence was made in the calculation of log(*K*) by fitting one concentration parameter for each of the 13 compound classes (section headings) in Table 1, the overall residual standard deviation was reduced from 0.0954 to 0.0682, which is essentially the minimum possible. When concentration dependence was added to the regression model, R^2 increased to 0.991, and the residual standard deviation decreased from 0.110 to 0.088. The overall effect of concentration, fitted last, was highly significant ($F = 45.7$, $df = 13, 1006$, $P \ll 0.0001$).

The 13 fitted concentration parameters could be combined into three, more general, class parameters without inflating residual

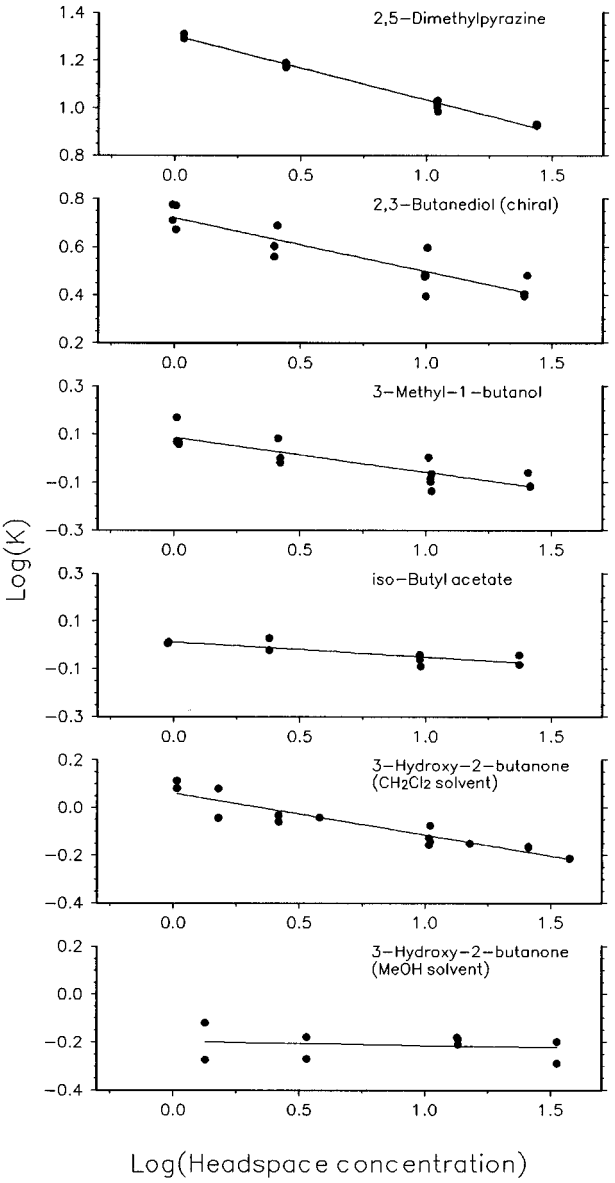

Figure 6. Relationship between log(*K*) and analyte headspace concentration for five compounds and two dilution solvents. Sampling temperature was always 25 °C.

variance: The nitrogen-containing compounds and the diols exhibited the greatest concentration dependence, and, overall, log(*K*) decreased by 0.31 (i.e., *K* decreased by a factor of 0.50) for each 10-fold increase in headspace concentration. The alcohols were intermediate, with *K* decreasing by a factor of 0.71 for each 10-fold concentration increase. Most remaining compounds (primarily the hydrocarbons, esters, aldehydes, and ketones) showed minor concentration dependence (but still highly significant, $P < 0.0001$); *K* decreased by a factor of 0.91 for a 10-fold increase in concentration. The relatively large standard errors for log(*K*) of the nitrogen compounds and diols (Table 1) were mostly due to the concentration dependence with these compounds.

Curiously, the concentration effect could vary with environment. The concentration effect was pronounced when methylene chloride was the solvent but was nearly absent when the analytes were in methanol solution (analysis mixture 6, Table 1). An example is 3-hydroxy-2-butanone, which was analyzed in both methanol and methylene chloride (Figure 6). The values of log(*K*) for the two solvents converged at the highest concentration of 3-hydroxy-2-butanone (25 μg in the sample bottle). The concentration effect was not included in the regression model for predicting *K* values (Table 2) because the effect appears to depend on what other compounds are present, it is still incompletely understood, and it is minor relative to other factors correlated to *K* such as retention index and sampling temperature.

Table 3. Comparison of Henry's Law Constants Determined Using SPME and Literature Values

compound	concn in aqueous phase (mg/mL)	Henry's law constant ($M\ atm^{-1}$) measd	lit.[6]
acetaldehyde	0.011	16	11.4, 13, 15
ethanol	0.097	171	200, 190, 160
1-propanol	0.11	98	150, 130, 160
ethyl acetate	0.013	7.5	7.4
2-methylpropanol	0.098	55	100
2-methylbutanol	0.10	71	71

Henry's Law Comparisons. The concentration of a volatile analyte in a dilute solution is proportional to its concentration in the headspace above (Henry's law), and there are many published values of Henry's law constants for aqueous solutions.[6] SPME offers a simple way to measure the headspace concentration in such a two-phase system, given that *K* is known for the analyte. Conversely, measurement of Henry's law constants with SPME and subsequent comparison to literature values can be used to check the accuracy of *K*. The results for six compounds used in our insect-attractant research are given in Table 3. Agreement between the measured and literature values is reasonably good, in view of the considerable variation among the literature values.

DISCUSSION

Overview of Results. Calibration data are presented for a commercial SPME device so that it can be used for routine quantitation of organic volatiles in the gas phase. The 71 standards used in the study were of diverse functionality and volatility and included many compounds commonly emitted from biological systems. The reported calibration factors (*K*), together with FID response factors, allow headspace concentrations to be calculated directly from SPME-GC peak areas. The *K* values and relative response factors in Table 1 could reasonably be used on any FID-equipped GC, once the absolute response factor for a standard such as pentadecane was determined. The *K* values would be valid with other types of GC detectors, but a different set of response factors would be required.

A regression model was developed that allows quantitation of many compounds beyond the original 71, as long as they belong to the studied functional classes, have a known GC retention index on a nonpolar column (ideally, on a DB-1 like that used here), and reach equilibrium with the fiber within the chosen sampling time. The regression model also allows any sampling temperature from 15 to 35 °C.

Practical Advantage of Equilibrated System. Determination of headspace concentration becomes more complicated if the SPME fiber cannot reach equilibrium for an analyte during the

(6) Betterton, E. A. Henry's Law Constants of Soluble and Moderately Soluble Organic Gases: Effects on Aqueous Phase Chemistry. In *Gaseous Pollutants: Characterization and Cycling*; Nriagu, J. O., Ed.; Wiley and Sons: New York, 1992.

Table 4. Estimated Limits of Detection by FID for Selected Analytes in Headspace Following SPME Sampling[a]

compound	*K*	minimum detectable headspace concentration ng/mL	ppb
acetaldehyde	0.015	5.6	3200
ethanol	0.087	0.76	400
2-hexanone	1.00	0.040	11
decane	5.89	0.0056	0.98
benzyl acetate	26.9	0.0015	0.24
ethyl decanoate	126	0.00036	0.044

[a] Based on 100 area units being the smallest detectable peak by FID (~30 pg of hydrocarbon).

sampling interval. If equilibrium is not established, *K* is a function of sampling time rather than a constant, and *K* would have to be measured for each sampling time used in the experiment. Predictions of *K* from the regression model would not be valid [*K* would be overestimated; see fitted values of log(*K*) in Table 1 for analytes that did not equilibrate]. These complications can be avoided in many cases by choosing the longest practical sampling time. In this study, 64 of 71 analytes equilibrated with the fiber within 30 min. Generally, compounds with a Kovats retention index on DB-1 of less than 1300 and carboxylic acids with an index less than 900 equilibrated within 30 min. It is possible that the larger compounds could equilibrate more rapidly with more vigorous mixing. Fibers with thinner or different polymeric coatings could also equilibrate more rapidly, but sensitivities (*K* values) would differ as well.

Dependence of *K* Values on Properties of Analyte. The calibration factors varied over nearly 4 orders of magnitude in this study. *K* was strongly related to Kovats retention index on a DB-1 column and to a lesser extent to analyte functionality. The relationship with Kovats index is not surprising because the SPME fibers and the DB-1 column have the same polymeric coating, poly(dimethylsiloxane), and similar absorption/desorption processes must occur for both.

In the regression model, relationships with functional group may be regarded as "fine tuning" once the general magnitude of *K* has been established with GC retention index. It is noteworthy that the SPME fibers are particularly sensitive for amines and other nitrogen-containing compounds and for alcohols and other hydroxy compounds. The nature of the interactions leading to this selectivity are not known, but the sensitivity of the fibers for these compound classes could be of considerable practical importance.

Limits of Detection. The limits of detection by SPME vary widely because SPME is far more sensitive to some compounds than to others. Table 4 gives example limits of detection, calculated on the basis of 30 pg of hydrocarbon providing the minimum usable FID signal. These values range from about 3 ppm in the headspace for acetaldehyde to about 0.04 ppb for tetradecane. Further reductions in minimum limits of detection are possible if detection methods more sensitive than the FID are used.

Temperature Dependence of *K*. The dependence of *K* on temperature observed in this study was similar to that described in a previous study.[3] However, a greater degree of change with temperature was observed here (decrease of 45% in *K* for each 10 °C increase in temperature) than in the earlier work (about 20% decrease for each 10 °C increase).

Because *K* is essentially an equilibrium constant, log(*K*) could be expected to have a linear relationship with the reciprocal of absolute temperature (Clausius−Clapeyron equation).[7] But since the temperature range was small in this study, the relationship between $1/T$ and T (with T expressed in Kelvin) is also nearly linear. Therefore, an almost linear relationship was anticipated between log(*K*) and temperature (either absolute or Celsius), and the Celsius temperature was used in the regression models.

Concentration Dependence. The observed concentration dependence was unexpected and introduces some uncertainty in the quantitation of certain analytes. This effect was most prominent for nitrogen-containing and hydroxy compounds that were dissolved in in methylene chloride yet was essentially absent when methanol was the solvent.

A model for the concentration effect is that the fibers have a small number of sites with especially high affinity for nitrogen compounds and alcohols and that this affinity operates in addition to the usual absorption mechanism. Once these sites become occupied, additional material (from higher concentrations) could enter the fiber only by normal absorption; the net effect would appear as concentration dependence of *K*. If there is an abundance of polar material present (such as methanol vapor), then these sites become saturated, and only the usual concentration-independent mechanism would operate.

This aspect of SPME needs further investigation so that appropriate adjustments can be applied to analyses. It is unknown whether water vapor could affect the absorption of small, polar analytes as methanol did or if competition between analytes in a mixture could skew results. A lower degree of analyte absorption from humid headspaces has been reported.[3] In any event, the observed effects of concentration and solvent competition on *K* values were small relative to the effect of retention index.

The data for concentration dependence do argue against analytes being adsorbed by bottle walls. Adsorption would probably be most obvious when analytes of high molecular weight were present in very small amounts, and the calculated *K* values in such cases would be lower than those for larger sample amounts. Instead, *K* values tended to be higher for the smaller analyte amounts or constant, rather than lower. The method for measuring the *K* values depended on negligible adsorption by bottle walls, and this situation appeared to exist.

Applicability of SPME. The wide range of fiber sensitivities to various compounds could affect the utility of SPME for some applications. The investigator should consider on a compound-by-compound basis whether SPME has sufficient sensitivity for the particular task at hand. Furthermore, the long equilibration time for the heaviest analytes could be a practical constraint. The investigator would have to decide whether sampling long enough to achieve equilibrium is possible. If not, the calibration factor must be determined specifically for the desired sampling time, and the less than maximum SPME sensitivity would have to be adequate for the needs of the study.

Nevertheless, SPME is ideal for analyzing many of the complex mixtures emitted from biological systems. The method produces an exceptionally flat and noise-free baseline, and there is no solvent peak that could mask some analytes. While the quantitative

(7) Daniels, F.; Alberty, R. A. *Physical Chemistry*, 3rd ed.; John Wiley and Sons: New York, 1966; Chapter 6.

precision of SPME may not be as high as that achievable by other methods, the simplicity, speed, low cost, and gentle treatment of analytes outweigh this disadvantage for our purposes. Furthermore, biological volatiles can easily vary over 2 or 3 orders of magnitude, and measurement to within, say, 25% can be very acceptable. It is likely that additional information, particularly with respect to the more polar analytes, will improve quantitation by SPME in the future.

This study dealt only with interactions between the headspace vapors and the fiber, but in practical situations one or more additional "phases" will be present, such as a fungal culture, a flower, or an insect, which emits volatiles into the headspace. In such cases, the transfer from matrix to headspace might even be the process of greatest interest. The present study does not address these multiphase systems, but it does discuss a tool for measuring the net effect, once the compounds of interest have reached the headspace.

ACKNOWLEDGMENT

Helpful discussions with Dr. Zhouyao Zhang and the competent technical assistance of Mr. Bruce Zilkowski are gratefully acknowledged.

Received for review August 13, 1996. Accepted November 7, 1996.[⊗]

AC960820N

[⊗] Abstract published in *Advance ACS Abstracts,* December 15, 1996.